# Novel 3-Benzoyl-2-piperazinylquinoxaline Derivatives as Potential Antitumor Agents



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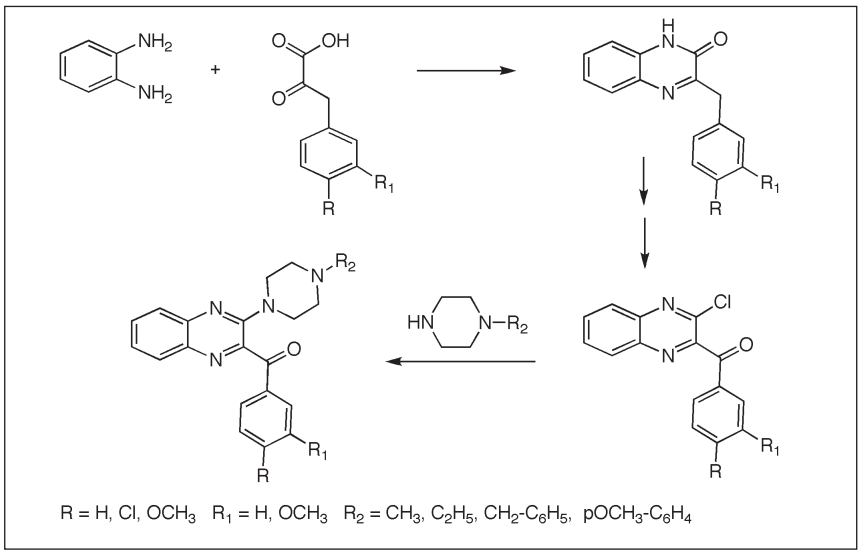


A series of new benzoylquinoxaline derivatives (**7-26**) was synthesized and evaluated for antitumor activity against a panel of 60 human cell lines at the NCI of Bethesda. Among the compounds which have passed the preliminary screening, compound **23** exhibited the best profile and growth inhibition activity at 100 - 10 μM. The compounds were then tested towards a folate-dependent enzymes bio-library including Thymidylate synthases enzymes and human Dihydrofolate reductase at 10 μM. The most of compounds exhibited a moderate inhibitory activity towards all or some of the enzymes tested with detectable inhibition constants ($K_i$) values in the range of 0.6-70 μM. Compounds **21**, **23**, **24** showed $K_i$ in the range of 10-38 μM against both hDHFR and hTS.

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Among all the folic acid derivatives that have been widely modified only two drugs, methotrexate (MTX) and tomudex (Figure 1), have emerged and are marketed as classical antifolic agents in anticancer chemotherapy, while among the non classical antifolates other two compounds, trimetrexate (TMQ) and piritrexim (PTX) (Figure 2), remain of interest as reference of antifolic activity but not in use as anticancer drugs because of their host toxicity [1-4].

According to the above-cited modifications methotrexate and tomudex are closely related to folic acid and maintain the glutamate moiety. In contrast trimetrexate and piritrexim contain a more lipophilic side chain reminiscent of well-known DHFR inhibitors trimethoprim (TMP) and pyrimethamine used as antimicrobial and antiprotozoal drugs.

As examples of modifications reported in the series of classical and non classical antifolate type compounds, Paglietti *et al*. have described more than three hundred quinoxaline derivatives referring to these classes on the ground that quinoxaline ring may act as bioisoster of pteridine or quinazoline ring.

From the biological screening at NCI several compounds were endowed with anticancer activity between 100 and 10 μM concentration [5-17] associated sometime with antifolic activity [11,15].

It was expected that the observed anticancer activity was due to the inhibition of key enzymes involved in DNA synthesis with folate-dependent activity. Among them Dihydrofolate reductase (DHFR) and Thymidylate synthase (TS), as part of the thymidylate synthase cycle. Most of the compounds were tested in free cells assays to identify the enzyme inhibition profile and some of them resulted in low micromolar inhibitors of DHFR [11,15]. These results prompted us to produce novel compounds with the aim to increase the DHFR and/or TS inhibitory activity and thus the anticancer action.

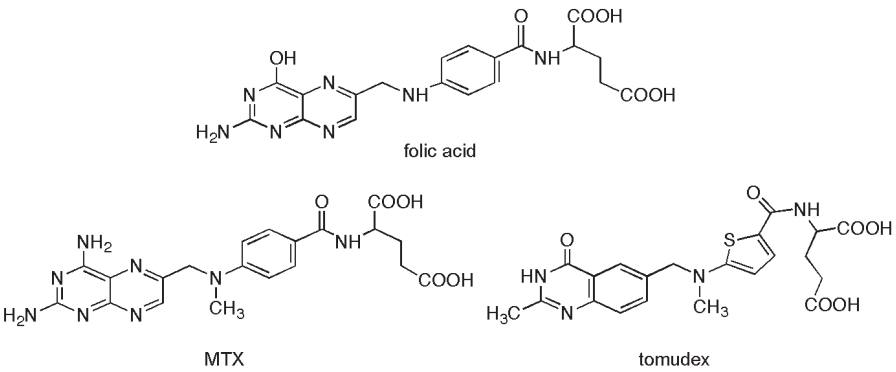



Figure 1. Folic acid and folate analogs with anticancer activity.

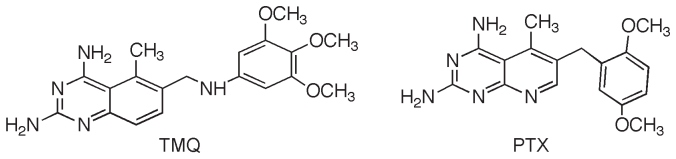



Figure 2. Folate analogs with antibacterial and antiparasitic activity.

In this note we have undertaken the preparation of compounds **7-26** (Figure 3; Scheme 1) in order to evaluate the influence of the substituents, which differ from those usually employed in the previous papers [5-17]. Quinoxaline does not bear any substituent in benzene counterpart while on the pyrazine ring in place of a phenyl group at position 3 we introduced a more flexible 3', 4'-disubstituted benzoyl moiety. At position 2 a $N^4$-substituted piperazine represents a novelty that in other cases led to interesting compounds endowed with anticancer activity [18].

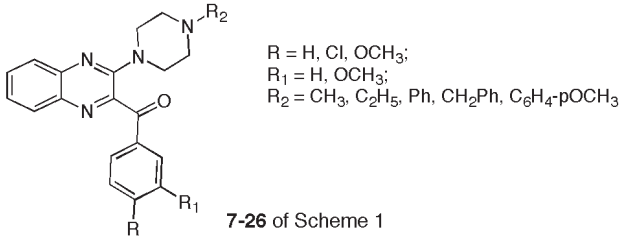


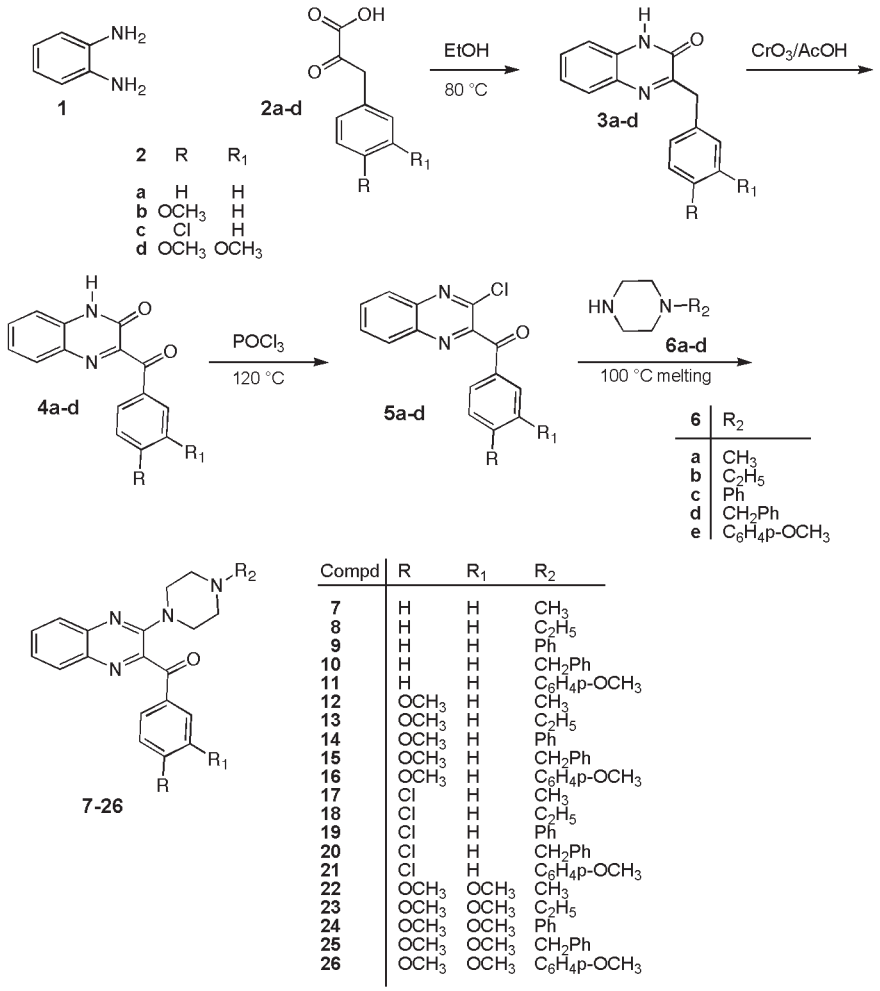

Figure 3. Compounds **7**-**26**, synthesized in the present work.

Additionally, it is well known that many folate-related compounds can show broader or species-specific inhibitory profiles [11,15], therefore, as a side screening rapid assay we explored the general antifolic inhibitory activity of the compounds against the folate-dependent enzyme bio-library based on TS, including also DHFR enzyme.

The preparation of compounds **7-26** was achieved according to the sequence of reactions of Scheme 1. 1,2-Diaminobenzene (**1**) and phenylpyruvic acid (**2a**), secondary cyclic amine (**6a-e**) were all commercially available. The acids **2b-d** were known and have been prepared according to literature [19,20,21]. Ring closure to quinoxalinones (**3a-d**) was obtained in good yields carrying out the condensation in ethanol at 90°C; compound **3a** was identical with that previously described by Romanenko *et al.* [22] while compound **3b** was reported by Pailer *et al.* [23]. The methylene bridge of **3a-d** underwent oxidation with chromic anhydride in acetic acid to 3-benzoylquinoxalinones (**4a-d**) in good yields. Compound **4a** was previously reported by Romanenko *et al.* [22], while compound **4b** was reported by Dahn and Nussbaum [24]. 3-Benzoyl quinoxalinones (**4a-d**) were converted into 2-chloro-3-benzoylquinoxaline derivatives (**5a-d**) by heating with an excess of $POCl_3$ at 120 °C. Of these intermediates, compound **5a** was known and reported in the reference [22], while compound **5b** appeared in other paper [24] without spectroscopic data. As we have extensively observed in other papers [5-17] thermal nucleophilic displacement of chlorine at position 2 or 3 of quinoxaline ring occurs easily by the secondary cyclic amine (**6a-e**) to give the desidered compounds **7-26** in fair to good yields (Table 1). Structure elucidation of the proposed structures came from the whole of both analytical and spectroscopical data (Tables 1 and 2).

All the compounds were tested against a TS-based bio-library composed of TS enzymes from different species such as *Enterococcus faecalis*, *Lactobacillus casei*, *Escherichia coli*, human Thymidylate synthases and human dihydrofolate reductase (Table 3). The $K_i$ values ranged within two orders of magnitude, 0.6 μM and 79

Scheme 1




μM. Compounds **7**, **8**, **11**, **14**, **23** showed $K_i$ values ranging between 7 and 46 μM towards EfTS (Table 3). The most interesting is compound **8** that is inactive at 10 μM, against the human enzymes, thus showing a specificity profile. Considering EcTS affinity profile, compounds **7**, **11-14** and **19**, showed some inhibitory activity in the range 20-74 μM. 10 compounds out of 17 showed some inhibitory activity against hDHFR, where compound **7** was the most active with $K_i$ value of 5 μM. The synthesized compounds did not inhibit very well hTS *i.e*. only 5 out of 17 showed some inhibitory activity and well above 20 μM. Due to solubility problems, compounds **10**, **25** and **26** couldn't be tested at 10 μM. In general we can consider that these compounds didn't show high affinity towards pathogenic enzyme TS, thus excluding LcTS as model enzyme, being compound **7** the most active one with a $K_i$ of 5μM against hDHFR.

Compounds **7-26** of Scheme 1 were submitted for *in vitro* anticancer evaluation at National Cancer Institute (Bethesda-USA) according to a well known screening program [25]. Only four of them (**13**, **17**, **23**, **24**) passed the preliminary three cell lines panel test and the results of their activity over 60 human tumor cell-lines are derived from dose-response curves and are presented in two different Tables (4, 5).

In Table 4 the response parameters (-log $GI_{50}$), (-log TGI) and (-log $LC_{50}$) refer to the concentration of the agent in the assay that produced 50% growth inhibition (GI), total growth inhibition (TGI) and 50% cytotoxicity (LC), respectively, and are expressed as mean graph midpoints.

In Table 5, we reported the activities of those compounds, which showed mean growth inhibition on 8 panel cell lines at μM concentration. From the data of Tables 4 and 5 it is evident that the most active was compound **23** with $GI_{50}$=15.1 μM and was selective against the colon panel cell line (6.9 μM) and significantly active at 10 μM over the most cell lines. Unfortunately, results show that this compound is also the most cytotoxic with $LC_{50}$=72.5 μM. Compound **13** was less active than **23** but exhibited

Table 1

Physical Properties of Compounds **7**-**26**

| Compd. | M p (°C) (*) | Yield% | Molecular formula (Molecular Weight) | Analysis (%) Calcd./Found C | H | N |
|---|---|---|---|---|---|---|
| 7 | 102-106 (c) | 59 | $C_{20}H_{20}N_4O$ (322.40) | 72.27 72.29 | 6.06 6.07 | 16.85 16.80 |
| 8 | 101-103 (a) | 40 | $C_{21}H_{22}N_4O$ (346.43) | 72.81 72.81 | 6.40 6.38 | 16.17 16.20 |
| 9 | 155-158 (a) | 95 | $C_{25}H_{22}N_4O$ (394.47) | 76.16 76.10 | 5.62 5.63 | 14.20 14.19 |
| 10 | Oil (g) | 73 | $C_{26}H_{24}N_4O$ (408.50) | 76.45 76.25 | 5.92 5.94 | 13.71 13.73 |
| 11 | 136-139 (f) | 59 | $C_{26}H_{24}N_4O_2$ (424.50) | 73.56 73.60 | 5.70 5.67 | 13.20 13.21 |
| 12 | 181-183 (c) | 43 | $C_{21}H_{22}N_4O_2$ (362.42) | 69.59 69.61 | 6.12 6.10 | 15.46 15.50 |
| 13 | 140-142 (c) | 45 | $C_{22}H_{24}N_4O_2$ (376.45) | 70.19 70.00 | 6.43 6.49 | 14.88 14.90 |
| 14 | 200-202 (b) | 70 | $C_{26}H_{24}N_4O_2$ (424.50) | 73.57 73.60 | 5.70 5.67 | 13.20 13.21 |
| 15 | 182-184 (e) | 66 | $C_{27}H_{26}N_4O_2$ (438.52) | 73.95 74.02 | 5.98 6.01 | 12.78 12.79 |
| 16 | 191-193 (e) | 40 | $C_{27}H_{26}N_4O_3$ (454.21) | 71.35 71.35 | 5.77 5.78 | 12.33 12.30 |
| 17 | 134-135 (d) | 57 | $C_{20}H_{19}ClN_4O$ (366.84) | 65.48 65.52 | 5.22 5.21 | 15.27 15.29 |
| 18 | 102-105 (h) | 38 | $C_{21}H_{21}ClN_4O$ (380.87) | 66.22 66.22 | 5.56 5.55 | 14.71 14.70 |
| 19 | 164-167 (a) | 77 | $C_{25}H_{21}ClN_4O$ (428.91) | 70.01 70.00 | 4.93 4.94 | 13.06 13.08 |
| 20 | 96-98 (f) | 41 | $C_{26}H_{23}ClN_4O$ (442.94) | 70.50 70.60 | 5.23 5.21 | 12.65 12.67 |
| 21 | 128-130 (f) | 40 | $C_{26}H_{23}ClN_4O_2$ (458.94) | 68.04 68.03 | 5.05 5.08 | 12.21 12.20 |
| 22 | 225-228 (d) | 45 | $C_{22}H_{24}N_4O_3$ (392.45) | 67.33 67.48 | 6.16 6.20 | 14.28 14.29 |
| 23 | 186-189 (d) | 28 | $C_{23}H_{26}N_4O_3$ (406.48) | 67.96 68.01 | 6.45 6.42 | 13.78 13.80 |
| 24 | 198-200 (a) | 60 | $C_{27}H_{26}N_4O_3$ (454.52) | 71.35 71.39 | 5.77 5.75 | 12.33 12.35 |
| 25 | 225-226 (g) | 57 | $C_{28}H_{28}N_4O_3$ (468.55) | 71.77 71.60 | 6.02 6.04 | 11.96 11.92 |
| 26 | 235-237 (g) | 27 | $C_{28}H_{28}N_4O_4$ (484.55) | 69.41 69.70 | 5.82 5.81 | 11.56 11.60 |

(*) = Purification procedure: (a) crystallized from ethanol; (b-h) flash cromatography (b) $CHCl_3$, (c) $CHCl_3/CH_3OH$ 98/2, (d) $CHCl_3/CH_3OH$ 95/5, (e) petrol ether (bp 40-70 °C)/ethyl acetate 85/15, (f) petrol ether (bp 40-70 °C)/ethyl acetate 8/2, (g) petrol ether (bp 40-70°C)/ethyl acetate 7/3, (h) ethyl acetate.

almost the same activity on Leukemia, melanoma, renal cancer cell lines. Compound **24** exhibited growth inhibition only on breast cancer cell lines. Comparing the tumor growth inhibition and the enzyme activity inhibitory profile for the four compounds evaluated *in vitro*, **23** and **24** showed the best enzyme inhibition profile against human enzymes, hTS and hDHFR. Moreover, compound **23** exhibited significant antifolate activity against bacterial EfTS, LcTS. Compound **13** showed a moderate enzyme inhibition activity towards hTS (Ki of 42 μM) and a $K_i$ of 0.60 μM against LcTS. Compound **17** showed some anticancer activity but was inactive against all the enzymes, thus it is not clear which inhibition mechanism is expected in this case.

In summary a series of new benzoylquinoxaline derivatives (**7-26**), structurally related to the nonclassical antifolates trimetrexate and piritrexim, in which the 5,8-dideaza- or 5-deaza-pteridine moiety was replaced by a quinoxaline ring, was synthesized. Evaluation of the data from both enzymatic and anticancer screening allow us to conclude that the observed anticancer activity can be due to an enzymatic inhibitory effect. In particular compounds **23** and **24**

Table 2
Spectroscopic (IR, UV, $^1$H NMR ) Data of Compounds **7**-**26**

| Compd | IR(nujol) ($\lambda\nu_{max}$ cm$^{-1}$) | UV(EtOH) ($\nu_{max}$ nm) | $^1$H NMR[**] $\delta_H$ (J in Hz) |
|---|---|---|---|
| 7 | 1650, 1580 | 207, 252, 377 | [A] 8.05 (d, 1H, H-8, J=7.4); 7.91 (d, 1H, H-5, J=7.4) 7.82-7.62 (m, 3H, arom); 3.56 (t, 4H, 2CH$_2$, J=4.8); 2.41(t, 4H, 2CH$_2$, J=4.8); 2.30 (s, 3H, CH$_3$) |
| 8 | 3400, 1650, 1600 | 207, 253, 382 | [A] 8.03 (d, 1H, H-8, J=7.4); 7.91 (d, 1H, H-5, J=7.4) 7.81-7.62 (m, 3H, arom); 7.56-7.42 (m, 4H, arom); 3.57 (t, 4H, 2CH$_2$, J=4.8); 2.44 (m, 6H, 2CH$_2$ and C*H*$_2$CH$_3$); 1.06 (t, 3H, CH$_2$ C*H*$_3$,, *J=7.2* ) |
| 9 | 1650, 1580 | 203,250 | [B] 8.02 (d, 1H, H-8, J=8.6); 7.86-7.60 (m, 3H, arom); 7.60-7.50 (m, 3H, arom); 6.95-6.78 (m, 4H, arom); 3.67 (t, 4H, 2CH$_2$, J=4.9);3.22 (t, 4H, 2CH$_2$, J=4.9) |
| 10 | 1650, 1580 | 207, 252 | [A] 8.03 (d, 1H, H-8, J=7.2); 7.90 (d, 1H, H-5, J=7.2); 7.80-7.76 (m, 2H, arom); 7.69-7.61 (m, 4H, arom); 7.55-7.29 (m, 6H, arom); 3.55 (t, 4H, 2CH$_2$, J=4.8); 3.49 (s, 2H, CH$_2$Ph); 2.43(t, 4H, 2CH$_2$, J=4.8) |
| 11 | 1640, 1580 | 203, 251 | [A] 8.04 (d, 1H, H-8, J=7.2); 7.93 (d, 1H, H-5, J=7.0); 7.84-7.64 (m, 4H, arom); 7.58-7.52 (m, 3H, arom); 6.90-6.84 (m, 4H, arom); 3.76 (s, 3H, OCH$_3$); 3.67 (t, 4H, 2CH$_2$, J=4.8); 3.05 (t, 4H, 2CH$_2$, J=4.8) |
| 12 | 1630-1600 | 378, 343, 328, 289, 274, 245, 217 | [A] 8.22 (dd, 1H, H-8, J=7.6 and 2.0); 7.88-7.80 (m, 2H, H-6,7); 7.70 (d, 1H, H-5, J=7.6 and 2.0); 7.40-7.20 (m, 4H, arom); 4.01 (s, 3H, OCH$_3$ ); 3.54 (t, 4H, 2CH$_2$, J=4.8); 2.72 (t, 4H, 2CH$_2$, J=4.8); 2.40 (s, 3H, CH$_3$) |
| 13 | 1630, 1600 | 378, 343, 329, 289, 274, 245, 217 | [A] 8.22 (d, 1H, H-8, J=7.8); 7.92-7.78 (m, 2H, H-6,7); 7.72 (d, 1H, H-5, J=6.8); 7.48-7.12 (m, 4H, arom); 4.01 (s, 3H, OCH$_3$); 3.55 (t, 4H, 2CH$_2$, J=5.0); 2.75 (t, 4H, 2CH$_2$, J=5.0); 2.54 (q, 2H, C*H*$_2$CH$_3$, *J=7.2*); 1.17 (t, 3H, CH$_2$ C*H*$_3$, *J=7.2*); |
| 14 | 1630, 1600 | 378, 343, 274, 246, 200 | [A] 8.24(d, 1H, H-8, J=7.8); 7.90-7.78 (m, 2H, H-6,7); 7.75 (d, 1H, H-5, J=7.8); 7.36 (d, 2H, H-2',6', J=8.2); 7.45-7.12 (m, 4H, arom); 7.05 (d, 2H, H-3',5', J=8.2); 7.98-7.90 (m, 1H, arom); 4.01 (s, 3H, OCH$_3$); 3.65 (t, 4H, 2CH$_2$, J=4.8); 3.49 (t, 4H, 2CH$_2$, J=4.8); |
| 15 | 1630, 1600 | 274, 245, 207 | [A] 8.25 (d, 1H, H-8, J=8.4); 7.87-7.80 (m, 2H, H-6,7); 7.70 (d, 1H, H-5, J=8.4); 7.50-7.22 (m, 9H, arom); 4.01 (s, 3H, OCH$_3$); 3.61 (s, 2H, CH$_2$); 3.53 (t, 4H, 2CH$_2$, J=4.8); 2.75 (t, 4H, 2CH$_2$, J=4.8); |
| 16 | 1620, 1600 | 378, 343, 328, 289, 274, 244, 217 | [A] 8.24 (d, 1H, H-8, J=8.4); 7.90-7.72 (m, 3H, arom); 7.52-7.20 (m, 4H, arom); 7.03 (d, 2H, H-3",5", J=9.2); 6.88 (d, 2H, H-2",6", J=9.2); 4.02 (s, 3H, 4'-OCH$_3$); 3.80 (s, 3H, 4"-OCH$_3$); 3.68 (t, 4H, 2CH$_2$, J=5.0); 3.40 (t, 4H, 2CH$_2$, J=5.0); |
| 17 | 1680, 1600 | 257, 210 | [A] 8.02 (d, 2H, H-2',6', J=8.8); 7.91 (d, 1H, H-8, J=8.2); 7.82 (d, 1H, H-5, J=8.2); 7.09 (m, 2H, H-6,7); 7.52 (d, 2H, H-3',5', J=8.8); 3.55 (t, 4H, 2CH$_2$, J=4.8,); 2.46 (t, 4H, 2CH$_2$, J=4.8,); 2.30 (s, 3H, CH$_3$); |
| 18 | 1680, 1600 | 258, 210 | [A] 8.02 (d, 2H, H-2',6', J=8.6); 7.90 (d, 1H, H-8, J=8.2); 7.80 (d, 1H, H-5, J=8.2); 7.67 (m, 2H, H-6,7); 7.51 (d, 2H, H-3',5', J=8.6); 3.56 (t, 4H, 2CH$_2$, J=4.8,); 2.54-2.40 (m, 6H, 2CH$_2$ and C*H*$_2$CH$_3$); 1.08 (t, 3H, CH$_2$ C*H*$_3$, *J=7.2*); |
| 19 | 1680, 1600 | 256, 204 | [A] 8.03 (d, 2H, H-2',6', J=7.8); 7.97 (d, 1H, H-8, J=8.4); 7.81 (d, 1H, H-5, J=8.4); 7.69 (t, 2H, H-6,7); 7.49 (d, 2H, H-3',5, J=8.2'); 7.28 (m, 2H, arom); 6.90 (m, 3H, arom); 3.68 (t, 4H, 2CH$_2$, J=5.0,); 3.21 (t, 4H, 2CH$_2$, J=5.0,); |
| 20 | 1680,1600 | 258, 209 | [A] 7.99 (d, 2H, H-.2',6', J=8.4); 7.89 (d, 1H, H-5, J=7.6); 7.60 (m, 2H, H-6,7); 7.47 (d, 2H, H-3',5', J=8.0); 7.40-7.22 (m, 5H, ,arom); 3.54 (t, 4H, 2CH$_2$, J=4.8,); 3.50 (s, 2H, CH$_2$); 2.45 (t, 4H, 2CH$_2$, J=4.8,); |
| 21 | 1670, 1590 | 256, 203 | [A] 8.02 (d, 2H, H-2',6', J=8.0); 7.92 (d, 1H, H-8, J=8.4); 7.83 (d, 1H, H-5, J=8.4); 7.69 (m, 2H, H-6,7); 7.52 (d, 2H, H-3',5', J=8.0); 6.90 (d, 2H, H-3",5", J=7.0); 6.62 (d, 2H, H-2",6", J=7.0); 3.76 (s, 3H, OCH$_3$); 3.67 (t, 4H, 2CH$_2$, J=4.8,); 3.08 (t, 4H, 2CH$_2$, J=4.8,); |
| 22 | 1630, 1610 | 358, 274, 218, 204 | [A] 8.20 (d, 1H, H-8, J=7.8); 7.80 (m, 2H, H-6,7); 7.73 (d, 1H, H-5, J=7.8); 7.46-7.25 (m, 3H, H-2',5',6'); 4.11 (s, 3H, OCH$_3$); 4.06 (s, 3H, OCH$_3$); 3.56 (t, 4H, 2CH$_2$, J=4.8,); 2.80 (t, 4H, 2CH$_2$, J=4.8); 2.44 (s, 3H, CH$_3$); |
| 23 | 1630, 1600 | 357, 274, 220, 205 | [A] 8.20 (d, 1H, H-8, J=7.8); 7.80 (m, 2H, H-6,7); 7.74 (d, 1H, H-5, J=7.2); 7.47-7.24 (m, 3H, H-2',5',6'); 4.10 (s, 3H, OCH$_3$); 4.06 (s, 3H, OCH$_3$); 3.54 (t, 4H, 2CH$_2$, J=4.6); 2.76 (t, 4H, 2CH$_2$, J=4.6); 2.56 (q, 2H, C*H*$_2$CH$_3$, *J=7.2*); 1.21 (t, 3H, CH$_2$C*H*$_3$, *J=7.2*); |
| 24 | 1630, 1600 | 354, 275, 251, 202 | [A] 8.21 (d, 1H, H-8, J=7.8); 7.80 (m, 2H, H-6,7); 7.75 (d, 1H, H-5, J=7.8); 7.52-7.25 (m, 5H, H-2',5',6' and 2H arom); 7.10-6.86 (m, 3H, arom); 4.11 (s, 3H, OCH$_3$); 4.06 (s, 3H, OCH$_3$); 3.65 (t, 4H, 2CH$_2$, J=4.6); 3.50 (t, 4H, 2CH$_2$, J=4.6), |
| 25 | 1630, 1610 | 355, 273, 203 | [A] 8.20 (d, 1H, H-8, J=7.8); 7.78 (m, 2H, H-6,7); 7.72 (d, 1H, , H-5, J=7.2); 7.45-7.22 (m, 8H, arom); 4.01 (s, 3H, OCH$_3$); 4.05 (s, 3H, OCH$_3$); 3.64 (s, 2H, CH$_2$); 3.52 (t, 4H, 2CH$_2$, J=4.8,); 2.75 (t, 4H, 2CH$_2$, J=4.8,); |
| 26 | 1600 | 253, 204 | [A] 8.22 (d, 1H, H-8, J=7.2); 7.81 (m, 2H, H-6,7); 7.76 (d, 1H, H-5, J=7.5); 7.46-7.25 (m, 3H, H-2',5',6',); 7.02 (d, 2H, H-3",5" , J=8.8); 6.88 (d, 2H, H-2"6" , J=8.8); 4.12 (s, 3H, OCH$_3$); 4.06 (s, 3H, OCH$_3$); 3.80 (s, 3H, 4"-OCH$_3$); 3.65 (t, 4H, 2CH$_2$, J=4.8,); 3.38 (t, 4H, 2CH$_2$, J=4.8,); |

[**]=Solvent: [A] $CDCl_3$; [B] $CDCl_3$-DMSO-$d_6$.

Table 3

Ki Values at 10μM Concentration of Compounds **7-26**

| Compd | EfTS | LcTS | EcTS | hDHFR | hTS |
|---|---|---|---|---|---|
| **7** | **12** | **33** | **74** | **5** | >190 |
| **8** | **15** | **37** | >190 | >190 | >190 |
| **9** | >190 | **70** | >190 | >190 | >190 |
| **11** | **46** | **33** | **59** | **29** | >190 |
| **12** | >190 | **1.6** | **20** | **16** | **29** |
| **13** | >190 | **0.6** | **32** | >190 | **42** |
| **14** | **7** | **14** | **32** | **18** | >190 |
| **15** | >190 | >190 | >190 | >190 | >190 |
| **16** | >190 | >190 | >190 | **12** | >190 |
| **17** | >190 | >190 | >190 | >190 | >190 |
| **18** | >190 | >190 | >190 | **79** | >190 |
| **19** | >190 | >190 | **62** | **38** | >190 |
| **20** | >190 | >190 | >190 | >190 | >190 |
| **21** | >190 | **53** | >190 | **38** | **27** |
| **22** | >190 | >190 | >190 | >190 | >190 |
| **23** | **8** | **10** | >190 | **10** | **28** |
| **24** | >190 | >190 | >190 | **38** | **20** |

Table 4

-log$GI_{50}$, -log$TGI$, -log$LC_{50}$ mean graph midpoints (MG-MID)[a] of *in vitro* inhibitory activity test for compounds **13**, **17**, **23**, **24** against human tumor cell lines[b].

| Compd | -log$GI_{50}$=μM | -log$TGI$ | -log$LC_{50}$=μM |
|---|---|---|---|
| **13** | 4.48 = 33.1 | 4.06 | 4.00 = 100 |
| **17** | 4.35 = 44.7 | 4.02 | 4.00 = 100 |
| **23** | 4.82 = 15.1 | 4.37 | 4.14 = 72.5 |
| **24** | 4.01 = 97.7 | 4.00 | 4.00 = 100 |

[a]MG-MID, mean graph midpoints; the average sensitivity of all cell lines towards the test agent; [b]from NCI.

Table 5

Mean growth-inhibition ($GI_{50}$) values in μM of compounds **13**, **17**, **23**, and **24**.

| Panel/Cell Line | **13** | **17** | **23** | **24** |
|---|---|---|---|---|
| | | $GI_{50}$ ( μM) | | |
| Leukemia | 26.0 | 40.7 | 11.2 | >100 |
| Non small Cell Lung Cancer | 29.0 | 44.0 | 15.1 | >100 |
| Colon Cancer | 34.0 | 40.7 | 6.9 | >100 |
| CNS Cancer | 39.0 | 64.5 | 10.9 | >100 |
| Melanoma | 24.0 | 30.2 | 24.0 | >100 |
| Ovarian Cancer | 69.1 | 72.4 | 16.9 | >100 |
| Renal Cancer | 24.0 | 40.0 | 20.9 | >100 |
| Prostate Cancer | 87.0 | 72.4 | 31.2 | >100 |
| Breast Cancer | 35.0 | 44.0 | 17.8 | 39.0 |

showed the best enzyme inhibition profile against human enzymes, hTS and hDHFR with a $K_i$ in the range of 10-38 μM.

## EXPERIMENTAL

Melting Points are uncorrected and were recorded on a Köfler or an electrothermal melting point apparatus. UV spectra are qualitative and were recorded in nm in ethanol solution with a Perkin-Elmer Lambda 5 spectrophotomer. IR spectra (Nujol mulls) were recorded with Perkin- Elmer 781 instrument. $^1$H NMR spectra were recorded at 200 MHz with a Varian XL-200 instrument using TMS as internal standard. Elemental analyses were performed at Laboratorio di Microanalisi, Dipartimento di Chimica, University of Sassari. The analytical results for C, H, and N were within ± 0,4% of the theoretical values.

General Procedure for Preparation of the 3-(3,4-R-benzyl)quinoxalin-2-ones **3a-d**.

A mixture of equimolar amounts (9.24 mmol) of 1,2-diaminobenzene (**1**) and the appropriate phenylpiruvic acid (**2a-d**) in ethanol (38 ml) was refluxed for 2 h. After cooling at 4 °C the crude product formed was collected to give the pure substances after recrystallization from ethanol.

Compound 3-benzylquinoxalin-2-one (**3a**) was obtained as described [22], in 64% yield, mp 197-199 °C (lit.[22]) 194-197°C).

Compound 3-(4-methoxybenzyl)quinoxalin-2-one (**3b**).

This compound was obtained in 61% yield, mp 198-199 °C (lit. [22] mp 198-199°C). Its $^1$H-NMR is now described for the first time :($CDCl_3$) δ: 12.65 (s, 1H, NH), 7.82 (dd, 1H, H8, J=8.2 and 2.8), 7.58-7.20 (m, 3H, H-5,6,7), 7.40 (d, 2H, H-2',6', J=8.6), 6.83(d, 2H, H-3',5', J=8.6), 4.22 (s, 2H, $CH_2$), 3.75 (s, 3H, $OCH_3$).

3-(4-chlorobenzyl)quinoxalin-2-one (**3c**).

This compound was obtained in 66 % yield, mp 226-227 °C. IR (nujol): 1630, 1580 cm$^{-1}$. UV (EtOH): 332, 282, 221, 202 nm. $^1$H-NMR ($CDCl_3$/DMSO-$d_6$) δ: 12.01 (s, 1H, NH), 7.75 (d, 1H, H-8, J=8.2), 7.43-7.35 (m, 3H, arom), 7.32-7.20 (m, 4H, arom), 4.20 (s, 2H, $CH_2$).

*Anal*. Calcd. for $C_{15}H_{11}ClN_2O$ (270.72) C, 66.55; H, 4.10; N, 10.35. Found: C, 66.60; H, 4.20; N, 10.44.

3-(3,4-dimethoxybenzyl)quinoxalin-2-one (**3d**).

This compound was obtained in 70% yield, mp193-197 °C. IR (nujol) : 3170, 1640, 1620, 1590 cm$^{-1}$. UV (EtOH) : 332, 281, 228, 204 nm. $^1$ H-NMR ($CDCl_3$/DMSO-$d_6$) δ: 12.18 (s, 1H, NH), 7.85 (d, 1H, H-8, J=7.8), 7.60-7.30 (m, 3H, arom), 7.28-7.09 (m, 2H, arom), 6.81 (m, 1H, arom), 4.23 (s, 2H, $CH_2$), 3,87 (s, 3H, $OCH_3$), 3.83 (s, 3H, $OCH_3$).

*Anal*. Calcd. for $C_{17}H_{16}N_2O_3$ (296.32) C, 68.91; H, 5.44; N, 9.45. Found: C, 69.15; H, 5.40; N, 9, 27.

General procedure for preparation of 3-(3,4-R-benzoyl) quinoxalin-2-ones (**4 a-d**)

To a mixture of the suitable 3-benzylquinoxalin-2-ones (**3a-d**) (4.00 mmol) in glacial acetic acid (30 ml), a 10% aqueous solution of chromic anhydride (5.3 ml) was added and then heated under stirring at 50 °C for 2 h. In the end water (22 ml) was added and the resulting mixture cooled at –20 °C overnight. The resulting precipitate was collected by filtration and washed with water to give white-yellow products, which displayed a single spot on tlc. Purification was accomplished on recrystallization from ethanol.

Compound 3-benzoylquinoxalin-2-one (**4a**) was obtained as described [22] in 68% yield: mp 258-260°C (lit [22] m.p.255-258 °C).

Compound 3-(4-methoxybenzoyl)quinoxalin-2-one (**4b**)

This compound was obtained as described [24] in 73% yield; mp 244-246°C (lit [24] mp 244-246 °C). IR (nujol): 1680, 1650, 1610 $cm^{-1}$ . UV (EtOH): 295, 296, 203 nm. $^1$H-NMR ($CDCl_3$/DMSO-$d_6$) δ: 12.65 (s, 1H, NH), 7.98 (d, 2H, H-8, J=8.8), 7.55 (d, 1H, H-5, J=8.6), 7.42-7.24 (m, 2H, H-6,7), 6.96 (d, 2H, H-3',5', J=8.6), 3.90 (s, 3H, $OCH_3$).

3-(4-chlorobenzoyl)quinoxalin-2-one (**4c**).

This compound was obtained in 73% yield, mp: 236-238 °C, IR (nujol): 3180, 1680, 1630, 1590 $cm^{-1}$. UV (EtOH): 258, 228, 204 nm. $^1$H-NMR ($CDCl_3$/DMSO-$d_6$) δ: 7.97 (d, 2H, H-2',6', J=8.2), 7.82 (d, 1H, arom., J=8.4), 7.70-7.25 (m, 3H, arom), 7.50 (d, 2H, H-3',5', J=8.2).

*Anal*. Calcd. for $C_{15}H_9ClN_2O_2$ (284.71) C, 63.28; H, 3.19; N, 9.84. Found: C, 63.07; H, 3.54; N, 10.01.

3-(3,4-dimethoxybenzoyl)quinoxalin-2-one (**4d**).

This compound was obtained in 59% yield, mp: 237-240 °C, IR (nujol): 3180, 1660, 1590 $cm^{-1}$. UV (EtOH): 285, 229, 204 nm. $^1$H-NMR ($CDCl_3$) δ: 12.18 (s, 1H, NH), 7.91 (d, 1H, H-8, J=8.0), 7.74 (s, 1H, H-2'), 7.56-7.26 (m, 4H, arom), 6.89 (d, 1H, arom, J=8.2), 3.98 (s, 3H, $OCH_3$), 3.96 (s, 3H, $OCH_3$).

*Anal*. Calcd. for $C_{17}H_{14}N_2O_4$ (310.31) C, 65.80; H, 4.55; N, 9.03. Found: C, 65,62; H, 4.80; N, 8.96.

General Procedure for Preparation of the 2-Chloro-3-(3,4-R-benzoyl) quinoxaline (**5a-d**).

A mixture of **4 a-d** (0.27 g, 9.15 mmol) and an excess of $POCl_3$ (2.3 ml, 24.70 mmol) was stirred under heating at 120 °C for 3 h. On cooling, the mixture was taken up with ice and the obtained solids were collected by filtration and washed with water to give the crude brown products, which were recrystallized from ethanol.

2-Chloro-3-benzoylquinoxaline (**5a**) was obtained as described [22] in 59% yield; mp 140-141°C, (lit. [22] mp 138-141°C) $^1$H-NMR ($CDCl_3$) δ: 8.18-8.10 (m, 2H, arom), 7.95-7.82 (m, 3H, arom), 7.72-7.45 (m, 4H, arom).

2-Chloro-3-(4-methoxybenzoyl)quinoxaline (**5b**).

This compound was obtained as described [24] in 70% yield; mp 120-121 °C (lit. [24] mp 114-115 °C) IR (nujol): 1680, 1620, 1600 $cm^{-1}$. UV (EtOH): 403, 352, 339, 291, 280, 248, 228 nm. $^1$H-NMR ($CDCl_3$-DMSO-$d_6$) δ: 8.22 (d, 2H, H-2',6', J=8.2), 7.80 (d, 1H, H-8, J=8.6), 7.72-7.37 (m, 3H, arom), 7.15 (d, 2H, H-3',5', J=8.2), 4.00 (s, 3H, $OCH_3$).

2-Chloro-3-(4-chlorobenzoyl)quinoxaline (**5c**).

This compound was obtained in 73% yield, mp: 143-146 °C, IR (nujol): 1680, 1640, 1580 $cm^{-1}$. UV (EtOH): 324, 243, 204 nm. $^1$H-NMR ($CDCl_3$) δ: 8.20-8.10 (m, 2H, arom), 7.84-7.86 (m, 4H, arom), 7.52-7.47 (m, 2H, arom).

*Anal*. Calcd. for $C_{15}H_8Cl_2N_2O$ (303.14) C,59.43; H, 2.66; N, 9.24. Found : C,59.40; H, 2.70; N, 9.19.

2-Chloro-3-(3,4-dimethoxybenzoyl)quinoxaline (**5d**).

This compound was obtained in 54 % yield, mp: 200-203 °C, IR (nujol): 1680, 1630, 1610 $cm^{-1}$. UV (EtOH): 375, 276, 221, 202 nm. $^1$H-NMR ($CDCl_3$) δ: 8.16 (d, 1H, J=8.4, H-8), 8.84 (d, 1H, H-5, J=7.8), 7.68-7.53 (m, 3H, arom), 7.48-7.18 (m, 2H, arom), 4.10 (s, 3H, $OCH_3$), 4.04 (s, 3H, $OCH_3$).

*Anal*. Calcd. for $C_{17}H_{13}ClN_2O_3$ (328.75) C, 62.11; H, 3.99; N, 8.52. Found: C, 61.89; H, 4.24; N, 8.42.

General Procedure for Preparation of the 2-(4-$R^2$-piperazinyl)-3-(3-R, 4-$R^1$-benzoyl)quinoxalines (**7-26**).

A mixture of one mole equivalent of chloroquinoxaline (8.4 mmol) (**5a-d**) and 3 mole equivalent (25.2 mmol) of the appropriate substituted piperazine (**6a-e**) was stirred under heating at 100 °C for 2.5 h. In all cases crude gummy products were formed and purified by recrystallization from ethanol (**8, 9, 19, 24**), or flash chromatography over silica gel eluting with chloroform (**14**); a mixture of chloroform/methanol in 98:2 ratio (**7, 12, 13**), in 95:5 ratio (**17, 22, 23**); a mixture petrol-ether/ethyl acetate in 85:15 ratio (**15, 16**), in 8:2 ratio (**11, 20, 21**), in 7:3 ratio (**10, 25, 26**); ethyl acetate (**18**).

Yields, mp values, analytical data are reported in Table 1 while spectroscopic data are reported in Table 2.

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